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CARDENOLIDES (HEART POISONS) IN THE PAINTED GRASSHOPPER *POECILOCERUS PICTUS* F. (ORTHOPTERA: PYRGOMORPHIDAE) FEEDING ON THE MILKWEED *CALOTROPIS GIGANTEA* L. (ASCLEPIADACEAE)

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Abstract.—The painted grasshopper *Poecilocerus pictus* F. feeds on the poisonous milkweed *Calotropis gigantea.* Cardenolide content in the various tissues of gravid females of this insect has been analysed and significant differences in the levels of cardenolides have been found in different tissues. The metathoracic scent gland, ovary and egg have been found to sequester higher concentrations of cardenolides. The accessory salivary system (reservoir), that is closely associated with the crop and the midgut, has been found to excrete the cardenolides, as evidenced from the analyses of the spittle emitted as droplets by satiated life stages.

At least fifteen species from eight orders of insects have been found to feed and reproduce on the plants of milkweed family Asclepiadaceae and Apocyanaceae (Von Euw et al., 1967, and Rothschild, 1972). They include several species of Lygaeidae (Duffey and Scudder, 1972; Isman, 1977; Moore and Scudder, 1986 and Dingle, 1991); Lepidoptera (Duffey, 1970, Brower et al., 1984 and Holzinger et al., 1992); Coleoptera (Rothschild, 1972; Hilker et al., 1993 and Eggenberger and Rowell-Rabier, 1993); aphids (Isman et al., 1977); scale insects (Rothschild et al., 1973); Cerambycidae (Duffey and Scudder, 1972); moths (Wink et al., 1990) and Orthoptera (Von Euw et al., 1967). It has been well established that these insects at some stage in their life cycle are able to sequester and store the heart poisons called cardenoides (cardiac glycosides) derived from their host plants and such insects are often brightly coloured (aposematic). It has been suggested that such compounds serve as chemical defense against vertebrate predators (Brower and Brower, 1964; Parsons, 1965; Von Euw et al., 1967, 1971; Reichesten et al., 1968, and Rothschild and Kellet, 1972).

In India, ten species of insects have been recorded on the milkweed *Calotropis gigantea* (L.), Asclepiadaceae (Pugalenthi and Livingstone, 1993). *Poecilocerus pictus* is a core species that feeds and breeds exclusively on the poisonous weed and utilizes the toxic cardenolides for its aposematic self-defence, (Livingstone and Pugalenthi, 1992). When the *Calotropis* bushes are completely denuded, the insects reach the adult stage and then migrate to adjacent supplementary host plants such as *Millingtonia hortensis* (Bignoniaceae), *Jatropha tanjorensis* (Euphorbiaceae), *Carica papaya* (Caricaceae) and *Moringa pterygosperma* (Moringaceae) which are totally devoid of cardenoides. Insects which are known to feed and breed on *Calotropis gigantea,* when reared on plants devoid of cardenoides, fail to develop and reproduce due to nutritional deficiency (Livingstone and Pugalenthi, 1992). However,

Table 1. *Poecilocerus pictus*: Cardenolides milieu in various tissues (mg/gram/ml. N-6 Mean ± SD). Means followed by the same letters are not significantly different at 5% level by DMRT.

	Fed on *C. gigantea*	Fed on *C. papaya*
Foregut	2.89 ± 0.72 ab	0.37 ± 0.04 a
Midgut	2.52 ± 0.56 ab	1.21 ± 0.15 b
Hindgut	1.44 ± 0.52 a	0.42 ± 0.99 a
Fatbody	1.84 ± 0.35 a	0.92 ± 0.33 b
Muscle	1.64 ± 0.28 a	0.86 ± 0.32 ba
Cuticle	1.39 ± 0.42 a	1.30 ± 0.25 b
Ovary	1.74 ± 0.67 a	1.48 ± 0.58 b
Eggs	1.69 ± 0.64 a	1.42 ± 0.75 b
Scent gland	3.89 ± 0.58 b	2.56 ± 0.46 c
Haemolymph	0.77 ± 0.21 c	0.47 ± 0.09 a
Spittle	1.21 ± 0.88 a	—

the mechanism of sequestration of the heart poisons in the various tissues of these insects, which are hooked to this poisonous weed from time immemorial, is only partially understood (Moore and Scudder, 1985, and Scudder et al., 1986).

MATERIALS AND METHODS

Adult females of *P. pictus,* collected from *C. gigantea* and *C. papaya* (which had fed for more than 45 days after having migrated from *C. gigantea*), were brought to the laboratory and immediately their haemolymph and spittle were collected in clean vials. Then the insects were kept for 30 minutes in a refrigerator at 5°C for immobilization. Such insects were dissected at 0° in insect ringer solution and their tissues such as muscle, fat body, ovary, eggs, foregut, midgut, hindgut, repugnatorial gland and cuticle collected separately.

Extraction and quantification of cardenolides present in the various tissues were carried out by adopting the modified method of Isman et al. (1977) and Eggenberger and Rowell-Rahier (1993). One hundred mg of each tissue from six individuals for each category was extracted with 5 ml of chloroform; methanol (2:1) for six hours and the extraction repeated. Then the two solvents were mixed, dried in hot water bath and the residue dissolved in a mixture of acetonitrile; water (50:50). High Performance Liquid Chromatography (HPLC) was used for cardenolide quantification. Twenty µl of each sample was manually injected and run by reverse phase HPLC (2 pump system; binary LC pump 250, Perkin Elmer) detector; UV diodearry detector 135, wave length: 219 nm, column; Macherey-Nagel cartridge, ca. 18.3 µm 4 × 30 mm, eluent; acetonitrile (Barker) and water (Merck) 50:50, flow rate 1 ml/min. The cardenolides were detected at 2.89 minutes. Ouabain (Sigma, USA) was used as the standard.

RESULTS

Table 1 represents the cardenolide concentration in various tissues of the grasshopper *P. pictus* collected from *C. gigantea* and *C. papaya.* It is clear that the cardenolides are not evenly stored in all the tissues. There is a wide difference in

the cardenolide content of the tissues of *P. pictus* collected from *C. gigantea* and *C. papaya* indicating that the cardenolide content of adult *P. pictus* is correlated with the cardenolide content of their respective diet tissue.

The metathoracic scent gland contains a much higher concentration of cardenolides, when compared with various other tissues in both categories of insects collected from *Calotropis* and papaya. The grasshopper discharges cardenolides from scent glands through the lateral openings of the scent gland, as a detterrent against its predators (Von Euw et al., 1967). It stores more cardenolides in the scent gland after having them transferred from various tissues through the haemolymph, even when it ceases to feed on plants containing cardenolides. It is noteworthy that the scent glands contain 2.56 mg/ml of cardenolides even 45 days after they were weaned from *Calotropis.*

The ovary and eggs of a maturing insect that continued to feed ad libitum, on *Calotropis* register higher concentration of cardenolides when compared with such estimates of insects that were fed on papaya leaves. However, the occurrence of a perceptible concentration of cardenolides in the ovaries and eggs of the second category of insects suggest that certain amount of cardenolides has already been sequestered in these structures and such concentrations do not decline drastically when the insect is weaned to the plants devoid of cardenolides.

In the case of insects collected from milkweeds the foregut registers a higher concentration of cardenolides and the midgut has relatively less quantity when compared with the foregut. This difference leads to the conclusion that the foregut may not absorb more cardenolides from the semidigested food and such cardenolides adhere to its membrane and enhance the cardenolide concentration in the foregut wall. It is also observed that the insects that are collected from papaya also contain residual cardenolides in its alimentary canal. Their midgut persists in revealing relatively higher concentration of cardenolides when compared with the foregut and hindgut. In the insects collected from milkweed the midgut record much high concentration of cardenolices than the hindgut. These results clearly indicate that the midgut is the main region of the alimentary canal through which cardenolides diffuse into the haemolymph.

Fat body, muscle and cuticle also serve as major reservoirs for cardenolides. In the insects collected from papaya, the concentration of cardenolides in the cuticle is not much less when compared with the fat bodies and muscles of the insects fed on *Calotropis.* It is probable that the cuticular cardenolides are not readily released.

Total absence of cardenolides in the spittle of the insects that are fed on papaya could be considered as evidence of selective excretory mechanism of the salivary gland. While cardenolide concentration in the spittle of the insect fed on *C. gigantea* remains high, the haemolymph records very low concentration of cardenolides, suggesting that the salivary gland excretes out the cardenolides from the foregut and midgut to which it is closely associated. Therefore, the excretion in the midgut, mainly in the crop, provides the source of the cardenolides in the saliva. The total absence of cardenolides in the food derived from non cardenolide plants, relieves the salivary gland of the function of excreting cardenolides and therefore the salivary gland of such insects are free from cardenolides. This also provides a very substantive evidence for establishing that these salivary glands function as the major excretory organs, right from the beginning of the digestive process in the foregut itself.

It is commonly observed that well fed, satiated life stages of this insect when allowed to feed continuously on *C. gigantea,* keep dropping spittle in copious amount as a post gorging behaviour. Such spittle when chemically analysed has very high concentration of cardenolides, confirming that salivary glands are among the principal organs excreting such heart poisons in the initial stage of absorption itself, in the foregut and midgut. The occurrence of cardenolides, in lesser concentration, in various other tissues of the insects that continue to feed on papaya could be considered as residual cardenolides, sequestered earlier by these tissues while feeding on *C. gigantea* during larval stages. However, there is no perceptible aposematic colour difference in the two groups of insects, namely the one that continues to feed on *Calotropis* and the other that feeds on papaya at the terminal stage.

DISCUSSION

Sequestration of cardenolides by insects has been documented only in a limited number of species, even though more than fifteen species of insects have been recorded feeding and reproducing on various species of the plants containing cardenolides. Isman et al. (1977) have analysed the cardenolide content of eight species of insects which feed on milkweeds in North America. According to their observations cardenoides concentration varied from species to species. The chrysomelid beetle *Oreina gloriosa* has been found to sequester a variety of cardenolides from their host plants during its various stages of life. The variations in the cardenolide concentrations among the adult individuals and larvae of this beetle is not based on age and size of the insect alone but on the ontogenetic modifications too (Eggenberger and Rowell-Rahier, 1993).

Cardenolide content in several species of the lygaeids, including *Oncopeltus fasciatus, Lygaeus kalmii* and *Spilostethus pandurus* have been thoroughly investigated by Isman et al. (1977), Duffey and Scudder (1972) and Rothschild (1972). These lygaeid bugs that sequester cardenolides are known to store in the fluid of their dorsolateral glands (62-270 μg of cardenolides per insect) in much greater concentrations, nearly 1000 times more than in any other body tissues such as the fat, muscle, metathoracic scent gland, cuticle and haemolymph (Duffey and Scudder, 1972).

Brower and his co-workers have analysed cardenolides content in *Danaus plexippus* both qualitatively and quantitavely. These butterflies sequester cardenolides from milkweeds, while they feed on it during the larval stage. The adults have been found to store cardenolides mostly in the wigns and use them as chemical defence (Brower and Glazier, 1975, and Brower et al., 1975, 1988).

Von Euw et al. (1967) reported earlier that in *Poecilocerus bufonius* the metathoracic scent glands remained as the major reservoir for such toxic cardenolides and the insect had adopted well to this poison and utilized it for its defence, development and reproduction. The LD 50 of Ouabain for this insect was found to be 200 mg/kg while in species of locusts it was found to be only 7 mg/kg.

The present study on the cardenolides concentration in various tissues of *Poecilocerus pictus* clearly illustrates that all the tissues store cardenolides in considerably high concentrations and the insect presents aposematic colouration. Metathoracic scent gland registers highest concentration followed by the fat bodies, muscles and

cuticle. However, haemolymph has relatively lesser concentration of cardenolides. The midgut, among other regions of the alimentary canal has greater concentration of cardenolides, suggesting that it is the main tissue responsible for cardenolides excretion and the salivary gland plays a major role in this function.

The present study therefore unequivocally establishes the fact that the physiology and biochemistry of this painted grasshopper have become highly specialized to utilize the heart poisons for its defence as well as for reproduction and growth. It is also clear that the cardenolides are excreted out through the digestive system and sequestered in the various tissues in varying levels of concentrations for utilization at appropriate stage of reproduction even when the source of nutrition is deprived of cardenolides.

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